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Control of Transcription of the Repressor Gene in Bacteriophage Lambda*

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Abstract. The rate of transcription of the structural gene for repressor (*cI* gene) in bacteriophage lambda is controlled by the amount of active repressor in the cell. When the (reversibly) thermolabile repressor in a bacterium lysogenic for λcI_{857} is inactivated by heat, the rate of repressor gene transcription immediately falls. If the repressor is renatured, synthesis of repressor messenger is immediately restored to a high rate. Control by the repressor of the rate of transcription of its own structural gene does not depend upon the expression of any other known gene because the control can be observed in an $N^- x^-$ lysogen.

Measurement of lambda repressor gene transcription was made possible by a two-step hybridization technique utilizing prehybridization to λdv plasmid DNA. Application of this technique also showed that the product of the *N* gene stimulates transcription of the *r* strand in the region of the *cII*, *O*, and *P* genes.

Infection of a cell with bacteriophage λ usually leads to one of two mutually exclusive timed sequences of gene expression. Either (1) the phage replicates many copies of its DNA, assembles new virus particles, and lyses the host cell, or (2) the phage replicates a few copies of its DNA, integrates one of those molecules into the chromosome of its host, and produces a repressor which blocks transcription of all but two genes of the virus. How does the infected cell choose between these two pathways, a choice which may resemble a single step in differentiation? The answer is not yet clear, but synthesis of the repressor appears to be a key event.

Genetic experiments of Eisen *et al.*[1] first suggested that the amount of active repressor in a defective lysogen was itself subject to some kind of control. In this paper we demonstrate that the concentration of active repressor regulates transcription of the repressor gene itself.

Results. Measurement of transcription of the λ repressor gene: The genome of phage λ is only about 1% of the genome of a lysogenic bacterium, and the repressor gene *cI* is only about 1% of the λ genome.[2] A special technique is therefore required to detect messenger RNA arising specifically from the *cI* gene. Pulse-labeled RNA extracted from λ lysogens is first hybridized to the DNA of a short, genetically defined fragment of λ, called λdv, which carries the repressor gene.[3] The λdv fragment, which contains one-eighth of the λ genome, has one endpoint between *N* and the V_2 operator, and the other between *P* and *Q* (Fig. 1). RNA complementary to this fragment is eluted and then hybridized

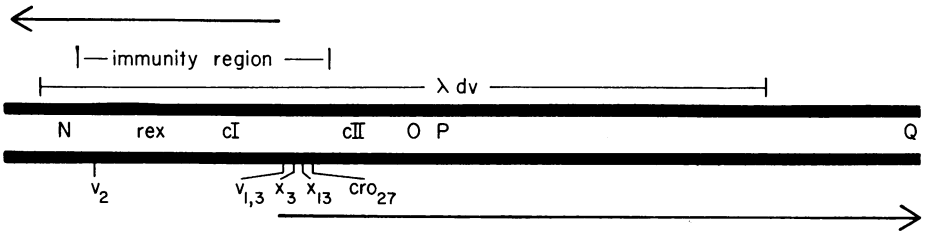


FIG. 1. Physical and genetic maps of the lambda immunity region (region of nonhomology with λ*imm*434) and neighboring phage genes[4-6] and of the λ*dv* plasmid[3]. Arrows indicate the direction of transcription.

to four DNA samples: (*i*) purified *r* strands of λ, (*ii*) purified *l* strands of λ, (*iii*) purified *r* strands of λ*imm*434 and (*iv*) purified *l* strands of λ*imm*434. λ*imm*434 DNA is homologous to λ DNA except for a substitution of nonhomologous 434 genes over a single short region, measuring 5.5% of the λ genome. This is called the immunity region,[4,5] and includes the *cI* gene. RNA which hybridizes to λ DNA, but not to λ*imm*434 DNA is, therefore, transcribed from the immunity region. We shall call this RNA "immunity RNA."

Immunity RNA is further analyzed by determining whether it hybridizes to the *r* or *l* strand of λ DNA; that is, whether it is transcribed to the right or to the left on the genetic map of λ (Fig. 1).

For these experiments we have used prophages which carry the cI_{857} mutation, and hence produce a temperature-sensitive repressor.[6,7] In these strains, when the temperature is raised from 30 to 42°C the repressor is denatured, immunity is lost, and there is a rapid increase in the transcription of the lytic genes.[8]

Transcription of the immunity region before, during, and after induction of a lysogenic bacterium: Line 1 of Table 1 shows the analysis of W3350 (λcI_{857})

TABLE 1. *Transcription of repressor gene before and after derepression.*

		Fraction of RNA hybridized to *l*-Strand			Fraction of RNA hybridized to *r*-Strand		
		of λ (1)	of λi[434] (2)	Immunity specific (1) − (2)	of λ (1)	of λi[434] (2)	Immunity specific (1) − (2)
W3350(λcI_{857})	30°C	2.8	0.2	2.6	<0.05	0.1	(0)
induced 1 min at	42°C	6.1	4.6	1.5	13.8	9.4	4.4
induced 5 min at	42°C	5.0	5.4	(0)	29.4	20.0	9.4
W3350(λ*NN*cI_{857}) at	30°C	2.6	0.4	2.2	0.8	0.1	0.7
Induced 5 min at	42°C	2.2	1.8	0.4	3.5	2.2	1.3
W3350(λ)	30°C	1.5	<0.05	1.5	<0.05	0.06	(0)
heated 5 min at	42°C	2.0	0.6	1.4	0.1	<0.05	0.1

All values in the table are to be multiplied by 10^{-4}. These values were calculated by dividing the radioactivity which hybridizes to the DNA filter by the total radioactivity added to the hybridization mixture in the first step. Results of duplicate hybridizations varied ±20%.

growing at 30°C. More than 95% of the λ-specific messenger RNA transcribed from the immunity region of a lysogen is transcribed from the *l* strand of the immunity region. This agrees with earlier findings.[9] Because the only genes which are known to function in the prophage state are *cI* and *rex*,[10] both of which are within the immunity region, it seems likely that these genes are transcribed

from the *l* strand, possibly as a single dicistronic messenger. Tentatively, we take the amount of *l* strand-specific immunity RNA as a measure of *cI* messenger RNA.

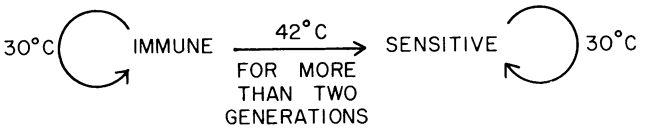
When the lysogen is induced by raising the temperature to 42°C, the rate of synthesis of *l* strand immunity RNA falls. By 1 min the net rate[11] has fallen to 50% (of that before derepression), and by 5 min to less than 5%. At the same time there is a large increase in the rate of transcription from both the *l* and *r* strands outside the immunity region (as indicated by the appearance of RNA that hybridizes to λ*imm*434), showing that the early genes located to the left and right of the immunity region have been derepressed. Thus, inactivation of the repressor protein to a degree sufficient to derepress the early genes also reduces transcription of the repressor gene itself. This, we suggest, reflects the operation of a system of control over expression of the repressor gene.

The product of gene *N* acts as a general positive regulator in λ.[10,12] A regulatory effect of the *N* product on transcription of the early lytic genes is evident in the data of Table 1. Comparison of the rate of synthesis of *r* strand RNA by λ*sus*$N^{-}{}_{7,53}$ and λN^{+} lysogens after derepression indicates that the rate of transcription of genes *cII*, *O*, *P* (i.e., *r* strand RNA which hybridizes to λ*dv* and to λ*imm*434 DNA) is reduced 9-fold by removal of the *N* product. In other experiments we have shown that *N* acts between *cro* and *O* to stimulate transcription.[13] The same experiment shows, contrariwise, that *N* product, eliminated by two amber mutations in gene *N*, has no effect on either the high level of transcription of the repressor gene (i.e., *l* strand immunity RNA) before induction, or the decrease in its transcription which follows heating to 42°C.

To verify that the effects of heating on the synthesis of λ messenger RNA are due to destruction of the (thermolabile) repressor, a lysogen for the c^{+} parent of λcI_{875} was analyzed. As shown in the lowest two lines of Table 1, heating did not decrease the rate of *l* strand immunity RNA synthesis in that lysogen.

Transcription of the immunity region in a prophage lacking the early genes: A deletion prophage has been isolated by Spiegelman[14] that possesses the region of the prophage from *int* through *x*, but is deleted for a segment commencing just to the right of the immunity region and extending through genes *cII*, *O*, *P*, *Q*, *R*, and for all the head and tail genes. This prophage carries the temperature-labile repressor mutation cI_{857}, and a second mutation, probably located to the left of gene *N*, which prevents the derepressed prophage from killing its host.

This strain can exist in either of two phenotypic states with respect to its immunity to λ and its sensitivity to T4*rII* superinfection. Sensitivity to T4*rII* is the test of *rex* function. Normally this strain is resistant to λ and to T4*rII* superinfection at 32°C. However, if it is grown at 42°C for many generations, and then returned to 32°C the strain becomes permanently sensitive to λ and T4*rII*. The cell is only phenotypically $[cI]^{-}$ and $[\text{rex}]^{-}$, because both genes can be recovered by superinfection. We say that such a defective lysogen is in the "sensitive" phenotypic state. The sensitive lysogen reverts at a low frequency to the $[cI]^{+}$ and $[\text{rex}]^{+}$ or "immune" phenotypic state demonstrating again that it has intact *cI* and *rex* genes. The genetic and physiological properties of this strain will be described in another publication[14].



The data presented in Table 2 show that more than 99% of the λ-specific RNA made by this defective lysogen when in the immune state is *l* strand immunity RNA. In this state, therefore, it resembles a normal lysogen both physiologically and in its pattern of transcription. Upon derepression by heating to 42°C the rate of synthesis of *l* strand immunity RNA decreases, just as it does in a nondeleted λcI_{857} lysogen. The amount of *r* strand RNA transcribed after derepression from genes outside the immunity region is small, confirming the genetic analysis of the strain that indicated that genes *cII*, *O*, and *P* had been deleted[14]. However, because of this deletion, it is now possible to see clearly that the rate of *r* strand synthesis within the immunity region increases after induction.

TABLE 2. *Repressor gene transcription in the immune and sensitive states.*

		Fraction of labelled RNA that hybridizes to *l*-Strand			Fraction of labelled RNA that hybridizes to *r*-Strand		
		of λ (1)	of λi^{434} (2)	Immunity specific (1) − (2)	of λ (1)	of λi^{434} (2)	Immunity specific (1) − (2)
Immune state	32°C	1.9	<0.01	1.9	0.01	<0.01	0.01
Transition 5 min at	42°C	1.6	1.2	0.4	0.9	0.1	0.8
Sensitive state	32°C	0.4	0.1	0.3	0.3	<0.05	0.3

All values in the table are to be multiplied by 10^{-4}. See Table 1 for details of calculation.

The defective lysogen was grown for many generations at 42°C, and then returned to 32°C. This treatment has been shown to convert this strain to the sensitive phenotypic state in which it does not make active repressor or T4*rII* restriction product. Analysis of the RNA synthesized by the defective lysogen in its sensitive state shows a four-fold lowering in the rate of synthesis of *l* strand immunity RNA compared to the same lysogen in its immune phenotypic state. Moreover, the rate of transcription outside the immunity region both of the *r* strand and of the *l* strand is higher in cells in the sensitive state than in the immune state, consistent with the idea that cells in the sensitive state lack repressor.

Effect of mutations in the immunity region on the regulation of the repressor gene: Two kinds of mutations have been investigated. The first is a mutation in the *x* region (x_3) which prevents the expression of the genes *cII*, *O*, and *P*, located in the operon governed by the $V_{1,3}$ operator (Eisen, H. A., personal communication). In addition to the mutation in the *x* region, the prophages studied in these experiments also carried double mutations in gene *N* to prevent expression of genes in the operon governed by the V_2 operator.[15–18] Data from these experiments are presented in the upper sections of Table 3. When the λcI_{857} *NNx* lysogen is derepressed by raising the temperature to 42°C, the rate of *l* strand immunity RNA synthesis decreased 3- to 5-fold just as it does in a nondefective (x^+) λcI_{857} lysogen. Note that the x_3 mutation prevents transcription of the *r*-strand, consistent with the genetic analysis (Eisen, H. A., per-

TABLE 3. *Repressor gene transcription in N, x, and cro mutants and the effect of renaturing the repressor.*

	Fraction of labelled RNA that hybridizes to *l*-Strand			Fraction of labelled RNA that hybridizes to *r*-Strand		
	of λ (1)	of λi^{434} (2)	Immunity specific (1) − (2)	of λ (1)	of λi^{434} (2)	Immunity specific (1) − (2)
Hfr H(λ*NNcI*$_{857}$) 30°C	3.5	0.1	3.4	0.5	<0.05	0.5
induced 5 min at 42°C	1.2	0.8	0.4	3.5	1.8	1.7
Hfr H(λ*NNcI*$_{857}x_3$) 30°C	1.7	0.1	1.6	0.2	<0.05	0.2
induced 5 min at 42°C	0.8	0.5	0.3	0.1	0.1	0
152(λ*cI*$_{857}$) 30°C	0.8	0.1	0.7	0.1	<0.05	0.1
induced 5 min at 42°C	1.0	0.9	0.1	22.6	12	10.1
152(λ*NNcI*$_{857}$*cro*$_{27}$$^-$) 30°C	1.8	0.1	1.7	0.3	<0.05	0.3
induced 5 min at 42°C	0.4	0.3	0.1	1.2	0.8	0.4
induced 5 min at 42°C and then renatured 5 min at 30°C	2.3	0.1	2.2	0.4	<0.05	0.4

The renaturation experiment was repeated with a prophage carrying the polar mutation x_{13} instead of the *cro*$^-$ mutation with identical results. All values in the table are to be multiplied by 10^{-4}. See Table 1 for method of calculation.

sonal communication). Kourilsky *et al.*[19] found a decrease in the synthesis of total (*l* strand plus *r* strand) immunity RNA after induction of a λ*NNx* lysogen, which is consistent with our finding that the synthesis of *l* strand immunity RNA is depressed.

Recently Eisen *et al.*[20] have identified a gene whose product appears to be necessary for a defective lysogen to establish a *stable* sensitive state after the destruction of repressor. This gene, called *cro*, maps within the immunity region, to the right of x_3 and x_{13}, and codes for a diffusible substance which can act through the cytoplasm (in *trans*). We have measured the synthesis of immunity RNA in a λ*cro*$^-$ lysogen and find again that *l* strand immunity RNA synthesis decreases after induction (Table 3).

Because *cI* gene transcription falls when repressor is inactivated in *NN*$^-$*x*$^-$ and *cro*$^-$ mutants, the decrease in transcription probably does not depend on the expression of any other known gene of λ, and, in particular, does not require the expression of the *cro* gene.

Repressor renaturation and the synthesis of repressor mRNA: One possible explanation for the decrease in the rate of *cI* transcription that follows destruction of repressor is that active repressor stimulates the synthesis of repressor mRNA. If this hypothesis is correct, then restoration of active repressor should restore repressor gene transcription, and the synthesis of *l* strand immunity RNA should rise to its former high level. The *cI*$_{857}$ repressor can be restored by shifting a culture from the denaturing temperature of 42°C to 30°C, thus allowing the repressor to renature.[7,21] Using this procedure we examined a lysogenic strain, in which the prophage is mutant in gene *N* and in the *cro* gene. This defective prophage was chosen to eliminate perturbations which might be produced by the *cro* product. As shown in Table 3, synthesis of *l* strand immunity RNA was depressed when the repressor was inactivated at 42°C. However, as predicted, when the repressor was renatured by returning the cells to 30°C there

was a large stimulation in the rate of *l* strand immunity RNA synthesis. In fact, after a 5-min period of renaturation, *l* strand RNA synthesis was slightly faster than the uninduced lysogen.

Discussion. We find that a λ lysogen transcribes a very limited portion of its prophage, namely, the *l* strand of the immunity region. This agrees with previous results,[9] and also with genetic experiments showing that only the *cI* and *rex* genes, which are located within the immunity region, are active in a lysogen.[10] Our experiments measure the rate of synthesis of *l* strand immunity specific RNA, which we take to be an estimate of the rate of transcription of the *cI* gene itself. Since this measurement includes transcription from the *rex* gene and, after induction, transcription in the region between V_2 and the left end of the immunity region, our results overestimate transcription of the *cI* gene.

Clearly the rate of synthesis of *cI* messenger RNA is specifically regulated. The rate falls when a lysogen is induced. A defective lysogen, capable of existing alternatively in an "immune" or a "sensitive" phenotypic state, synthesizes *cI* messenger at a high rate in the immune state, and at a low rate in the sensitive state. What λ gene products does the regulation require? The defective lysogen WG6 is deleted for a segment commencing just to the right of the immunity region and extending through genes *cII*, *O*, *P*, *Q*, *R* and for all the late genes[14]. Nevertheless, this strain exhibits the same decrease in rate of synthesis of repressor messenger after induction as does a normal lysogen. Therefore, the products of all the genes deleted in WG6 are ruled out as responsible for the observed control. The product of gene *N* is necessary for the expression of early genes to the left of the immunity region.[15–18] An NN^- lysogen also shows the decrease in *cI* transcription following induction. This seems to leave only the immunity region itself as a possible locus for a *cI* regulating gene. Mutations in the *x* region prevent expression of early genes transcribed from the *r* strand, and located to the immediate right of the immunity region (Eisen, H. A., personal communication). Again λNN^-x^- exhibits the induced decrease in *cI* transcription. Therefore, it seems unlikely that the decrease in repressor mRNA synthesis requires the synthesis of any phage product. Rather we favor the hypothesis that the repressor protein stimulates transcription of its own structural gene.[22] The idea that repressor may stimulate its own synthesis was first proposed by Eisen *et al.*[1] to account for the existence of a sensitive phenotypic state in NN^-O^- and NN^-P^- lysogens.

It was possible to verify one prediction of this hypothesis, namely, that renaturation of heat-denatured repressor should restore the high rate of synthesis of *l* strand immunity RNA. Preliminary kinetic experiments indicate that the rate is completely restored within 1 min of growth at 30°C.

Though sufficient to explain an initial decrease in rate of *cI* messenger RNA synthesis following derepression, this hypothesis is not sufficient to explain dominance of the sensitive over the immune state as observed by Eisen *et al.*,[20] and Calef and Neubauer.[23] Recently Eisen *et al.*[20] have identified a gene, *cro*, which is essential to maintain a defective λcI_{857} lysogen in the sensitive state. We have found, however, that the initial decrease in the rate of repressor mRNA synthesis does not depend on the *cro* product. Preliminary experiments indicate

that the *cro* product may be necessary to stabilize transcription of repressor gene at the low rate. This would be consistent with the physiological studies of Eisen *et al.*,[20] Calef and Neubauer,[23] and Spiegelman[14]. The mechanism of action of the *cro* product is not clear at present. It could act directly on *cI* transcription, or indirectly through the effect of repressor on *cI* transcription.

Materials and Methods. Medium: Cultures were grown in minimal-salts medium supplemented with 0.2% glucose and 0.2% acid-hydrolyzed casein. For growth of the defective lysogen WG6, medium was supplemented with deoxycytidine (10 μg/ml).

Pulse-labeling: RNA was pulse-labeled by adding 1 mCi of [5-^{3}H]uridine (20–30 Ci/mmol, obtained from Schwarz) to a 20-ml culture growing exponentially. After one-twentieth of a generation, unlabeled uridine was added to a concentration of 50 μg/ml and NaN_3 to 0.01 M, and the cells were chilled in solid CO_2–ethanol.

Lysogenic bacterial strains: (*a*) *Deletion prophage:* The defective lysogen, WG6, carries a deletion prophage on the F′*trp* episome. The prophage in WG6 is described in the text, and will be described in more detail in another publication[14]. (*b*) *Bacteria strains:* 152rif^r:*rec*$^-$ rifamycin^R*su*$^-$, and W3350:*gal*$_1$$^-$*gal*$_2$$^-$*su*$^-$ were lysogenized with the appropriate phage: λ*ind*$^-$, λ*ind*$^-$*cI*$_{857}$ (provided by A. D. Kaiser) or with λ*ind*$^-$*cI*$_{857}$*sus*$N_{7,53}$ (provided by E. R. Signer). The following lysogens were given to us by H. Eisen: 152rif^r(λ*ind*$^-$*cI*$_{857}$*sus*$N_{7,53}$*cro*$_{27}$):Hfr H:su$^-$ (λ*ind*$^-$*cI*$_{857}$*sus*$N_{7,53}$) and Hfr H (λ*ind*$^-$*cI*$_{857}$*sus*$N_{7,53}x_3$) Strain KM424 carries the λ*dv* plasmid, and was given to us by K. Matsubara.

RNA extraction: The cells were lysed according to the procedure of Godson and Sinsheimer.[24] The cells were treated with lysozyme and EDTA, and then lysed by adding Brij 58, $MgSO_4$, sodium deoxycholate, and DNase. After 5 min at 0°C, the lysate was extracted three times with phenol and the RNA was precipitated with ethanol.

Isolation of λ*dv* DNA: λ*dv* DNA was isolated as supercoils from strain KM424 in collaboration with K. Matsubara.[3]

Isolation of λ DNA strands: Denatured λ or λ*imm*434 DNA was banded in CsCl in the presence of poly UG.[25] The separated strands were sedimented in an alkaline sucrose gradient and then self-annealed.[26] The purity of the strands was tested by hybridization to λ RNA of known polarity. 98% of the RNA made by a λTII mutant was found to hybridize to the *l* strand.[9] 95% of the RNA made at late times after induction of λ$^+$ hybridized to the *r* strand.[9]

DNA filters: (*a*) λ*dv* DNA supercoils were denatured by heating at 66°C for 10–60 min in 0.5 N NaOH. After neutralization the DNA was loaded onto filters in 6 × SSC (SSC: 0.015 M sodium citrate—0.15 M NaCl). The DNA was fixed to the filter by heating at 80°C for 2 hr in an evacuated chamber. (*b*) The purified, separated strands were loaded directly onto the filters and fixed as above.

Two-step hybridization procedure: (*a*) *First step:* hybridization to λ*dv* DNA. The procedure is that of Gillespie and Spiegelman[27] as modified by Champoux.[28] After hybridization the filters (containing 0.7 μg DNA) were washed and the RNA was eluted by heating the filter to 95°C in 0.01 × SSC—0.01 M Tris, pH 7.1. The RNA solution was treated with DNase, and then heated to 95°C for 10 min.[28] (*b*) *Second step:* hybridization to the separated strands of λ or λ*imm434*. The eluted RNA was hybridized to a second DNA filter by the same procedure as in the first step. The filters contained 1 μg of DNA. After the hybridization, the filters were washed and treated with RNase. The radioactivity of a filter that had no DNA fixed to it was taken as a blank. This value (roughly equal to $10^{-5}\times$ the input radioactivity) was subtracted from that of the DNA-containing filters. In these experiments 50–90% of the RNA that hybridized to the λ*dv* filter in the first step rehybridized to the λ($l + r$) strands in the second step.

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